

Antifungal effects of the aqueous and ethanolic leaf extracts of *Echinophora platyloba* and *Rosmarinus officinalis*

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(Received: 5 January 2016; Revised: 20 February 2016; Accepted: 2 March 2016)

Abstract

Background and Purpose: In traditional medicine, herbal products still remain the principal source of pharmaceutical agents. The present study aimed to investigate the antifungal effects of *Echinophora platyloba* and *Rosmarinus officinalis* extracts on *C. albicans* species.

Materials and Methods: The aqueous and ethanolic leaf extracts of *E. platyloba* and *R. officinalis*, collected from the mountainous regions of Iran, were screened in terms of antimicrobial activity against *C. albicans* strains, using the agar well diffusion method. The minimum inhibitory concentration was determined by the microtitration technique.

Results: Overall, the results showed that the leaf extracts of *E. platyloba* and *R. officinalis* had strong antimicrobial activities. Also, based on the findings, *R. officinalis* leaf extracts exhibited higher antimicrobial activity. The ethanolic leaf extracts of *E. platyloba* and *R. officinalis* showed good antimicrobial activity against *C. albicans* strains. However, the aqueous extracts did not show any major activities against the tested *C. albicans* strains. On the other hand, the ethanolic extracts exhibited major antimicrobial properties against *C. albicans* strains. The highest minimum inhibitory concentration was reported in *E. platyloba* leaf extracts.

Conclusion: The present results indicated some advantages of *E. platyloba* and *R. officinalis* leaf extracts, which could be applied for the treatment of microbial infections.

Keywords: Antimicrobial effect, Extract, *Echinophora platyloba*, *Rosmarinus officinalis*, Iran

➤ How to cite this paper:

Sepehri Z, Javadian F, Khammari D, Hassanshahian M. Antifungal effects of the aqueous and ethanolic leaf extracts of *Echinophora platyloba* and *Rosmarinus officinalis*. Curr Med Mycol. 2016; 2(1): 30-35. DOI: [10.18869/acadpub.cmm.2.1.30](https://doi.org/10.18869/acadpub.cmm.2.1.30)

Introduction

Since ancient times, medicinal plants have been known for their antimicrobial activities. However, the importance of these plants was not acknowledged until the early 20th century [1]. Some plant species, used in traditional medicine, are available in mountainous areas at relatively lower costs compared to modern pharmaceutical drugs [2]. In fact, many secondary metabolites, which are produced by plants, comprise an important source of fungicides, bacteriocides, pesticides, and many pharmaceutical drugs.

In traditional medicine, herbal products still remain the principal source of pharmaceutical agents [3, 4]. The study of herbal treatments has a long history in traditional Iranian medicine. *Rosmarinus officinalis* L. or rosemary, belonging to the Lamiaceae family, is a pleasant-smelling perennial shrub which grows in several regions around the world [5]. It is a well-known and valuable medicinal

herb which is widely used in pharmaceutical products and traditional medicine as a digestive, tonic, astringent, diuretic, and diaphoretic agent; in addition, it is used for the treatment of urinary ailments [6].

In addition, *Echinophora* is a ten-species genus of Apiaceae family, with four species endemic to Iran, i.e., *E. orientale*, *E. sibthorpiana*, *E. cinerea*, and *E. platyloba*; this plant is called “Khousharizeh” or “Tigh Touragh” in Farsi. *E. platyloba* is widely used as a food seasoning and edible vegetable in western and central parts of Iran. The locals add this plant to pickles and tomato paste as an antifungal and antimicrobial preservative [7]. Also, this plant is used as a stomach tonic, diuretic, and anti-cancer agent [8].

The antifungal effects of *E. platyloba* extracts on fungi such as *Trichophyton rubrum*, *Microsporum gypseum*, *M. canis*, and *C. albicans* have been

confirmed in the literature [9]. The essential oil of this plant contains distillation para seaman (43.34%), α - phellandrene (88.21%), and α -pinene (31.3%) [8]. Also, *E. platyloba* and *Foeniculum vulgare* (fennel) are traditionally used for menstrual disorders.

E. platyloba belongs to the Umbelliferae family and consists of four species, including *E. cinerea*, *E. platyloba*, *E. orientalis*, and *E. sibthorpiana*. The two mentioned herbal species are exclusive to Iran [10]. *E. platyloba* species are known by different local names such as “Khoshariz”, “Tigh Touragh”, “Tigh Masti”, “Khoshandar”, “Kouzang”, “Tanghez”, and “Khousharouz”. This plant is a pasture plant used for flavoring food, cheese, and yoghurt [7].

In a previous study by Sadraei et al. [10], *E. platyloba* extracts could reduce ileum contractions in rats. Moreover, the antifungal effects of *E. platyloba* have been depicted on a number of common dermatophytes [7]. Recently, fluconazole-resistant *C. albicans* strains and intrinsically resistant *Candida* species, such as *C. glabrata* and *C. krusei*, have emerged in immunocompromised patients receiving therapy or prophylactic treatment [11-13]. Also, Mahbobi et al. by comparing the anti-*Candida* effects of *E. platyloba* with amphotericin [14, 15] indicated the effectiveness of this plant in the treatment of *C. albicans* infections.

In the present study, the two discussed plants were collected from Zabol, Iran, and their antifungal effects on *C. albicans* were assayed. So far, there have been no reports on the antifungal activity of these two local plants. Therefore, the aim of this study was to investigate the antimicrobial activity of aqueous and ethanolic extracts of *E. platyloba* and *R. officinalis* leaves collected from the mountainous regions of Iran against *C. albicans* strains.

Materials and Methods

Plants

The extracts of *E. platyloba* and *R. officinalis* were prepared, using a rotary device. *E. platyloba* and *R. officinalis* were collected from the mountainous regions of Iran (Zabol) and then chopped. After collecting the plants, they were rinsed with water and chopped for microbial tests. Then, they were dried for the preparation of plant extracts in the shadow.

Preparation of aqueous, ethanolic, and ethyl acetate extracts

The powder of *E. platyloba* and *R. officinalis* (50 g) was prepared over 30 min, using boiling water, ethanol, and ethyl acetate (250 ml). Afterwards, the decoction was filtered and then freeze-dried to obtain the aqueous extracts [16]. For

extract preparation, 10 g of the dry plant powder was placed in half-liter erlens, containing 100 ml of 96% ethanol and water.

The content of the flasks was mixed at room temperature for 24 h by the shaker at 130 rpm and then filtered using Whatman paper No. 2. The solvent was separated from the extract by the rotary device, using a vacuum pump (vacuum distillation). The obtained extracts were weighed, dissolved in dimethyl sulfoxide (DMSO) solvent, and maintained in the refrigerator at 4°C for further use [16].

Isolation of *C. albicans*

The gynecologists collected a total of 20 positive vaginal samples, using a sterile swap and a falcon tube. Yeast identification was performed through Gram staining, macromorphology, micromorphology, germ tube test, and evaluation of growth characteristics of *Candida* species on a CHROMagar *Candida* medium.

Fluconazole susceptibility test

The susceptibility tests were carried out by the M44-A2 method. Fluconazole 25 mcg disks, produced by Centro de Controle e Produtos para Diagnósticos Ltd. (CECON), were used in the research [17].

Minimum inhibitory concentrations (MICs)

The MICs of echinocandin aminocandin (AMN) and comparators against each isolate were determined according to CLSI M27-A2 document. The cell count was standardized using a hemocytometer. The suspension was adjusted in RPMI-1640 medium and buffered with MOPS [3-(N-morpholino) propanesulfonic acid] (Hardy Diagnostics, Santa Maria, CA) to $2-5 \times 10^3$ CFU/ml and $0.4-5 \times 10^4$ CFU/ml for *Candida* and filamentous fungi, respectively. Microdilution plates were incubated at 35°C over 24 h for *Candida* species [17].

Statistical analysis

The mean values were analyzed, using MINITAB Release 13.20 Program. One-way analysis of variance (ANOVA) was performed to determine the most effective plants and the most sensitive organisms.

Results

The antimicrobial activities of the aqueous and ethanolic leaf extracts of *E. platyloba* and *R. officinalis* are presented in Table 1. The aqueous leaf extracts of *E. platyloba* showed no major activity

Table 1. Antifungal activity and minimum inhibitory concentration (MIC; mg/ml) of *Echinophora platyloba* and *Rosmarinus officinalis* leaf extracts against *Candida* species

<i>C. albicans</i> strains	MIC/MFC (<i>E. platyloba</i>)	MIC/MFC (<i>E. platyloba</i>)	MIC/MFC (<i>E. platyloba</i>)	MIC/MFC (<i>R. officinalis</i>)	MIC/MFC (<i>R. officinalis</i>)	MIC/MFC (<i>R. officinalis</i>)
	Aqueous	Ethanollic	Ethyl acetate	Aqueous	Ethanollic	Ethyl acetate
1	-/-	100/200	50/50	-/-	50/100	25/50
2	-/-	100/200	50/50	-/-	50/100	25/50
3	-/-	25/50	12/5.25	-/-	25/50	12/5.25
4	-/-	25/50	25/50	-/-	100/200	50/200
5	-/-	150/300	100/200	-/-	25/50	25/50
6	-/-	150/300	50/100	-/-	50/100	50/100
7	-/-	250/500	100/200	-/-	150/150	50/100
8	-/-	100/200	50/100	-/-	50/100	12/5.25
9	-/-	100/200	50/100	-/-	50/100	25/50

No activity

against the tested *C. albicans* strains. However, the ethanolic extracts exhibited good inhibitory effects against the majority of *C. albicans* strains. The results showed that the MIC of *E. platyloba* against *C. albicans* was 25 ppm; therefore, this fungus could be inhibited with this concentration of the plant extract.

The results of the present study showed that *E. platyloba* extracts could inhibit the growth of *C. albicans*, as indicated by the increased MIC. The MIC values for all *C. albicans* strains are presented in Table 1. As shown in this table the growth of the most strains can be inhibited at 25 ppm concentration of plant extract. (Table 1). Based on the findings, the aqueous leaf extract of *R. officinalis* was not active against *C. albicans* strains (Table 1). However, the ethanolic extracts showed good inhibitory effects against most of *C. albicans* strains.

According to the findings, the lowest MIC of *R. officinalis* against *C. albicans* was 25 ppm, while the highest MIC against *C. albicans* strains was 150 ppm (Table 1). The results showed that the MIC of ethyl acetate *E. platyloba* extract against *C. albicans* was 12.5 ppm, while the highest MIC was 100 ppm against *C. albicans* strains.

The lowest MIC of ethyl acetate *R. officinalis* extract against *C. albicans* was 12.5 ppm, while the highest MIC was 50 ppm (Table 1). The findings revealed that the lowest minimum fungal concentration (MFC) of ethanolic *E. platyloba* extracts was 50 ppm against *C. albicans*, while the highest MFC was 500 ppm. The lowest MFC of ethyl acetate extract was 25 ppm, whereas the highest MFC was 200 ppm against *C. albicans* strains (Table 1).

Discussion

In pharmaceutical sciences, the main goal of the majority of recent studies on natural plant extracts is to introduce scientific and acceptable agents to

replace antimicrobial drugs. These agents can be particularly used to fight resistant strains which might be difficult to manage, even with the use of multidrug therapies [13, 14].

In a previous study by Abdulaziz et al. [18], the diameters were higher in thyme essential oil (42.4±6.5) in comparison with rosemary essential oil (11.8±2.8). The serial two-fold dilutions of the tested essential oils showed that both oils exhibited antifungal activities even at very low concentrations. Moreover, in a study by Matsuzaki et al. [19], the MICs of α -pinene, 1,8-cineole, and camphor, as the major components of rosemary chemotypes (used with Tween 80), were 0.63, 5.0, and 5.0 μ l/ml against *C. albicans*, respectively. Also, the antifungal activity increased 4-8 times by adding Tween 80. The MFCs of α -pinene and 1,8-cineole were similar to the MICs against *C. albicans*, while the MFC of camphor was twice as high as the MIC. Also, α -pinene showed the lowest MIC and MFC among rosemary chemotypes.

In a study by Tavassoli and Emamdjomeh [20], the antimicrobial activity of rosemary leaf extract against *Leuconostoc mesenteroides*, *Lactobacillus delbrueckii*, *Saccharomyces cerevisiae*, and *C. krusei* (*Issatchenkia orientalis*) was determined by MIC measurements. The results indicated that the rosemary extract had a stronger inhibitory effect against the bacteria. The MIC values for both *Leuconostoc mesenteroides* and *Lactobacillus delbrueckii* ranged between 1.5 and 1.75 mg/ml.

Furthermore, in a study by Kilanc et al. [21], the effect of rosemary extracts was examined against food-borne pathogenic bacteria, i.e., *Staphylococcus aureus*, *Bacillus cereus*, *Campylobacter jejuni*, and *Salmonella infantis*. The Gram-positive strains were much more sensitive to rosemary extracts. Based on the agar dilution method, the MICs against *S. aureus* and *B. cereus* ranged between 0.078 and 5.0 mg/ml,

whereas for *S. infantis*, the values were within the range of 5.0-10.0 mg/ml.

E. platyloba is one of the four endemic species in Iran used in food products [10]. The antimicrobial and antifungal effects of *E. sibthorpiana* have been reported in the literature [15]. In addition, Sadraei et al. [10] showed that *E. sibthorpiana* extracts could reduce rat ileum contractions *in vitro*. The results demonstrated the essential constituents of *E. platyloba*, i.e., α -flinders (09.32%), limonene (28.16%), cymene (75.10%), α -pinene (79.9%), varactyl alcohol (79.3%), and β -myrcene (65.2%). Overall, the antibacterial effects of *E. platyloba* on *S. aureus* were found to be significant. The MIC and MFC for these two plants against *C. albicans* were 16 ppm and 63 ppm, respectively [22].

The results of a previous study by Zarali showed that α -phellandrene (08.24%), followed by resin (32.16%), varactyl alcohol (12.9%), and α -pinene (30.8%) were the major components of *E. platyloba* essential extracts. Based on the MIC test results, *E. coli* had the lowest inhibition (6.4 mg/ml) and the minimum bactericidal concentration (75.18 mg/ml) [23]. In a study by Ali et al. [24], the essential oils from the flower, leaf, and stem extracts of *E. lamondiana* were analyzed by gas chromatography-flame ionization detection and gas chromatography-mass spectrometry (GC-MS). The major components of essential oils from the flower, leaf, and stem of *E. lamondiana* were δ -3-carene (61.9%, 75.0%, and 65.9%, respectively), α -phellandrene (20.3%, 14.1%, and 12.8%, respectively), and terpinolene (2.7%, 3.3%, and 2.9%, respectively).

The flower and leaf essential oils, as well as terpinolene, exhibited biting deterrent activities similar to 25 nmol/cm of N,N-diethyl-meta-toluamide (DEET; 97%) against *Aedes aegypti* L. and *Anopheles quadrimaculatus* Say. The compounds (+)- δ -3-carene and (R)- α -phellandrene, as well as water-distilled essential oils, were significantly less repellent than DEET. Also, in a study by Avijan et al. [25], the appearance of drug-resistant *C. albicans* and the adverse effects of chemical agents raised the researchers' interest in *E. platyloba* as one of the four native species in traditional Iranian medicine.

The results of the present study showed the potent synergistic effects of *E. platyloba* ethanolic extract, itraconazole ($P < 0.01$), and fluconazole ($P < 0.001$), while the antagonistic effects of *E. platyloba* ethanolic extract, clotrimazole, and miconazole were reported against the clinical isolates of *C. albicans*. Also, in a study by Fraternali et al. [26], the chemical composition and antimicrobial activity of the essential oils obtained from the flowering aerial parts and ripe

fruits of *E. spinosa* L. (Apiaceae family) were analyzed by GC/MS in central Italy.

In the mentioned study, the major constituents of the oil from the aerial parts of the plant were β -phellandrene (34.7%), myristicin (16.5%), delta-3-carene (12.6%), α -pinene (6.7%), and α -phellandrene (6.2%). Also, p-cymene (50.2%), myristicin (15.3%), α -pinene (15.1%), and α -phellandrene (8.1%) were the major components in the oil of ripe fruits. The extracted oils showed good antimicrobial activity against *Clostridium difficile*, *C. perfringens*, *Enterococcus faecalis*, *Eubacterium limosum*, *Peptostreptococcus anaerobius*, and *C. albicans* with MICs of 0.25, 0.25, 0.25, 2.25, and 0.50%, respectively. The corresponding values for the aerial parts and ripe fruits were 0.13, 0.13, 0.13, 0.13, 2.25, and 0.50%, respectively.

In a previous study by Hashemi et al. [27], the chemical analysis of *E. platyloba* via GC/MS showed that ocimene (26.51%), 2,3-dimethylcyclohexa-1,3-diene (9.87%), α -pinene (7.69%), and gamma-dodecalactone (5.66%) were the dominant components of the essential oil. The main constituents of the methanolic extract were o-cymene (28.66%), methanol (8.50%), α -pinene (7.42%), and gamma-dodecalactone (5.20%). The essential oil showed strong antimicrobial activity against the tested bacteria, whereas the methanolic extract almost remained inactive against Gram-negative bacteria.

The most sensitive bacteria to the essential oil and extracts of *E. platyloba* DC were *L. monocytogenes* and *S. aureus*. The MICs of the essential oil against *L. monocytogenes* and *S. aureus* were 6250 and 12500 ppm, respectively. Also, the MIC of the methanolic extract against *S. aureus* and *L. monocytogenes* was 25000 ppm. According to a study by Gavanji and Larki [28], the propolis extract with MIC₉₀ and MFC of 39 and 65 μ g/ml showed the highest antifungal activities, respectively, compared with other studied extracts. Also, the extracts of *Allium cepa* and *Thymus vulgaris* (MFC= 169 and 137 μ g/ml, respectively) showed the least significant effects on the fungi. In addition, nystatin and amphotericin B yielded better effects on the tested fungi in comparison with other studied extracts on *C. albicans*.

Conclusion

Based on the results of the present study, the leaf extracts of *E. platyloba* and *R. officinalis*, collected from the mountainous regions of Iran, showed strong antimicrobial activity, although

the observed activity was more significant in *R. officinalis* leaf extracts. The ethanolic leaf extracts of *E. platyloba* and *R. officinalis* exhibited good activity against *C. albicans* strains. However, the aqueous extracts showed no major activity against the tested *C. albicans* strains. The ethanolic extracts presented high antimicrobial properties against *C. albicans* strains, and the highest MIC was reported with the leaf extracts of *E. platyloba*.

Acknowledgments

This study was supported by Zabol University of Medical Sciences, Zabol, Iran.

Author's contribution

All authors equally contributed to the study design, study implementation, statistical analysis, and manuscript writing.

Conflicts of interest

The authors declare no conflicts of interest.

Financial disclosure

This study was supported by Zabol University of Medical Sciences, Zabol, Iran.

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